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gp100 gene. This fragment was digested with *EcoRV* and *XhoI* and cloned into *EcoRV/XhoI* digested NVQH6MC5#10 generating plasmid C5H6MELgp100 #5 which contains the gp100 gene linked to the H6 promoter.

The gp100 gene in plasmid C5H6MELgp100 #5 was sequenced using  
 5 custom primers. A 65bp deletion was found in this clone and shown to be present in pCDNA3-gp100. Plasmid PCR11-gp100 was used in PCR with oligonucleotides MELgp05(5'-CCC-ATC-TGG-CTC-TTG-GTC-3') (SEQ.ID.NO. 115) and MELgp13 (5'-TGA-CAT-CTC-TGC-CAG-TGT-GGT-3') (SEQ.ID.NO. 116) to generate a 0.6kb fragment. This fragment was digested with *Bam*HI and  
 10 *Asp*718 and ligated to a 6.5kb *Asp*718/*Bam*HI (partial) fragment from C5H6MELgp100 #5 generating plasmid C5H6MELgp100 which contains the entire gp100 gene under the control of the H6 promoter.

Pre-existing plasmid pC5H6MELgp100 was used as template for site directed mutagenesis of the two CTL epitopes beginning at amino acids 209 and  
 15 280, respectively. Primers used were:

209-A

GCT CAG CCT TCA CCA TTA TGG ACC AGG TGC CTT TCT CC  
 (SEQ.ID.NO.117)

209-B

20 GGA GAA AGG CAC CTG GTC CAT AAT GGT GAA GGC TGA CG  
 (SEQ.ID.NO.118)

280-A

GAG CCT GGC CCA GTC ACT GTT CAG GTG GTC CTG CAG CC  
 (SEQ.ID.NO.119)

25 280-B

GCC TGC AGG ACC ACC TGA ACA GTG ACT GGG CCA GGC TC  
 (SEQ.ID.NO.120)

A section containing the modified epitopes was sequenced and isolated as a 440 bp *Nco*1/*Mlu*N1 fragment. This fragment was ligated into

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pC5H6MELgp100 digested with *Nco*I and *Mlu*NI, creating a plasmid with the complete gp100 with the modified epitopes 209-2M and 280-9V.

Sequence data revealed a G to C substitution at bp# 10, changing a.a. # 4 from a Valine to a Leucine. This was corrected by PCR using the following primer pair;

MEL25

GCT CCG GGA TCC CCG GCG ATG GTA GAC AGT CAC TTC CAT CGT GTG  
TGT GCC CAG CAT TG (SEQ.ID.NO.121)

MEL27

ATC GCG ATA TCC GTT AAG TTT GTA TCG TAA TGG ATC TGG TGC TAA  
AAA GAT GCC TTC TT (SEQ.ID.NO.122)

MEL25 changes bp# 549 from a C to a G destroying the unique *Nco*I site for easier screening. It does not change the amino acid.

The resulting PCR fragment was digested with *Bam*HI and *Eco*R5 and replaced the equivalent fragment correcting the error. The resulting plasmid is pC5gp100-M which is shown in Figure 3 (SEQ.ID.NO.123).

#### Genetic modification of the recipient:

Recombination between donor plasmid pC5gp100M and ALVAC(2) rescuing virus generated recombinant virus vCP1584, which contains the vaccinia H6 promoted modified human gp100 in the C5 locus.

#### EXAMPLE 3

##### Screening for the identification and purification of recombinant organisms:

The aspects of screening for the identification and purification of a recombinant organism of the present invention is set out below.

(1) Plaque purification was done using *in situ* plaque hybridization (Piccini *et al.*, Methods of Enzymol. 153:545 (1987)) was used to identify recombinant viruses and to demonstrate purity of final virus preparations. *In situ* plaque hybridization analysis was performed with radiolabelled probes specific for the gp100 construct (a 580 bp fragment) and the C5 insertion locus.

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(2) Restriction analysis: Viral genomic DNA was isolated from cells infected with ALVAC parent or ALVAC(2)-gp100M (vCP1584). The genomic DNA was digested with restriction endonucleases (*HindIII*, *Pst* I or *Bam*HI). The resultant DNA fragments were fractionated by electrophoresis through an agarose gel and visualized by ethidium bromide staining. The insertion of the mod gp100 expression cassette at the C5 locus was confirmed.

(3) Immunoprecipitation analyses: These were performed using radiolabeled lysates derived from uninfected HeLa cells or cells infected with either ALVAC parental virus, ALVAC-gp100 (vCP1465) or ALVAC(2)-gp100M (vCP1584) as described previously (Taylor *et al.* J. Virol. 64:1441 (1990)). Briefly, HeLa cell cultures were infected at an m.o.i. of 10 pfu/cell in methionine-free media supplemented with [35S]-methionine (35uCi/ml). At 18 hrs. post infection, cells were lysed. Immunoprecipitation was performed using a rabbit anti-gp100 serum (AZN-LAM, received from M. Schreurs University of Nijmegen, Netherlands). Immunoprecipitates were fractionated on a 10% SDS-Polyacrylamide gel. The gel was fixed and treated for fluorography with 1M Na-salicylate for 1/2 hr. The dried gel was exposed to Kodak XAR-2 film to visualize the protein species. Results with anti-gp100 demonstrate expression of gp100 in ALVAC-gp100 infected HeLa cells but not for parentally infected cells. (See Figure 6)

(4) Western Blot. HeLa cells were infected for 18 hours at a multiplicity of 10 pfu/cell with ALVAC(2)-gp100M (vCP1584), ALVAC-gp100 (vCP1465) or ALVAC. Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose. The blot was incubated with AZN-LAM (1/5000 dilution) followed by HRP conjugated swine anti-rabbit utilizing the enhanced chemiluminescence (ECL) detection method (Amersham). Results demonstrate expression of full length gp100 in ALVAC-gp100 and ALVAC(2)-gp100M infected cells. (See Figure 7).

(5) Plaque immunoscreen analysis. This was performed on vCP1584 material to determine phenotypic stability of the virus upon passaging. The

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phenotypic stability of production batch material of ALVAC-gp100M (vCP1584) was analyzed by an immunological plaque assay which measures expression of the inserted genes at the plaque level. The assay utilizing permeabilized cells for detection of intracellular as well as surface expression of Hgp100mod was chosen  
5 for this test.

Test and control reagents (ALVAC(2)-gp100M (vCP1584) and ALVAC standard and ALVAC-gp100M, respectively) were plated on CEF monolayers under agarose at dilutions resulting in 40-200 plaques per 60 mm dish. 120 hours after incubation at 37°C, the infected monolayers were processed by plaque  
10 immunoassay for detection of internal expression of gp100M. Positive and negative plaques were counted for test and control samples. The primary antibody used was Monoclonal Anti-HMB50 at 1:800 dilution. A secondary antibody used was horse radish peroxidase (HRP)-conjugated rabbit anti-mouse antiserum diluted 1:500.

15 The result of analysis of internal expression of Human modified gp100 by individual plaques produced by (vCP1584) is presented in Table 1.

The result demonstrates that 98.7% of the plaque population of ALVAC-gp100M is expressing gp100M indicating that ALVAC-gp100M is phenotypically stable.

20 Results of the plaque immunoscreen analysis demonstrate that ALVAC(2)-gp100M is phenotypically stable with respect to expression of gp100.

(6) Nucleotide sequence analysis. This was performed on vCP1584 to validate the nucleotide sequence of the H6-promoted melanoma gp100M cassette. The sequence analysis revealed no nucleotide differences relative to the  
25 expected sequence, thus no mutations were introduced during the production of vCP1584. In order to carry out this analysis, a pool of plasmid clones containing a 2.2 kb PCR-derived fragment (encompassing the H6-promoted melanoma gp100M cassette), generated from vCP1584 genomic DNA was used.

pBS/1584 was generated by pooling 9 positive clones obtained by the  
30 ligation of a 2.2 kb PCR fragment (containing the H6-promoted melanoma

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gp100M cassette from vCP1584), into pBS-sk-(Stratogene). The 2.2 kb PCR fragment was derived from vCP1584 genomic DNA with the oligonucleotide primers, IDC5-1 and IDC5-2 (Figure 5). The nucleotide sequence of the oligonucleotide primers used to sequence pBS/1584 are listed in Figure 5.

5 **EXAMPLE 4**

This example provides results from injection in cynomolgus monkeys of modified gp100 molecules.

**Methods and Experimental Design**

**Test System**

10 Cynomolgus monkeys (*Macaca fascicularis*) purpose bred animals.

Supplier: Siconbrec "Simian Conservation Breeding & Research Center Inc.", Fema Building, 44 Gil Puyat Avenue Makati, Metro Manila, Philippines.

Number of animals in the study: 12 (6 males and 6 females).

Age at initiation of treatment: 26 to 38 months.

15 - Body weight range at initiation of treatment (day -1):

- males: 1.73 to 2.34 kg

- females: 1.71 to 2.65 kg.

**Animal Husbandry**

- Housing: one air-conditioned room;

20 - temperature: 19 to 25°C (target range),

- relative humidity: >40%

- air changes: minimum 8 air changes per hour,

- lighting cycle: 12 hours light (artificial)/12 hours dark.

25 - Caging: animals were housed singly in stainless steel mesh cages (approximately 540 x 810 x 760 mm).

- Diet: expanded complete commercial primate diet (Mazuri diet, Special Diet Services Ltd., Witham, Essex, CM8, 3AD, Great Britain) analyzed for chemical and bacterial contaminants.

Quantity distributed: 100g diet/animal/day.

30 In addition, animals received fruit daily (apple or banana)

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Animals were fasted for at least 16 hours before blood sampling for clinical laboratory investigations and before necropsy.

- Water: drinking water *ad libitum* (via bottles).

- 5   - Contaminants: no known contaminants were present in diet or water at levels which might have interfered with achieving the objective of the study.

#### **Pre-Treatment Procedures**

- Animal health procedure: all animals received a clinical examination for ill-health on arrival and a veterinary clinical examination during the acclimatization period.

- 10   - Acclimatization period: at least 3 weeks between animal arrival and start of treatment.

#### **Experimental Design**

- Allocation to treatment groups was performed during the acclimatization period using a random allocation procedure based on body weight classes.

- 15   - Animals were assigned to the treatment groups shown in Table 2. The dose levels administered were shown in Table 3.

#### **Administration of the Test/Control Articles**

##### Group 1 and 2 Animals

- 20   - Method of administration: injection in the left inguinal lymph node. Animals were lightly anaesthetized before each administration by an intramuscular injection of ketmine hydrochloride (Imalgene® 500 - Merial, Lyon, France). The same lymph node was injected on each occasion (left side). Each injection was followed by a local disinfection with iodine (Vétédine® - Vétquinol, Lure, France).

##### 25   Group 3

- Route: subcutaneous.
- Method of administration: bolus injection using a sterile syringe and needle introduced subcutaneously. Four injection sites were used followed by a local disinfection with iodine (Vétédine® - Vétquinol, Lure, France). Animals were
- 30   also lightly anaesthetized before each administration by an intramuscular

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injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France) in order to be under the same conditions as groups 1 and 2 animals.

Four injection sites in the dorsal cervical/interscapular regions were used as shown in Table 4.

5 **ELISPOT Analysis**

An ELISPOT assay was used in order to assess the cell mediated immune response generated in the monkeys in the various treatment groups. In particular, an ELISPOT IFN $\gamma$  assay was used in order to measure IFN $\gamma$  production from T lymphocytes obtained from the monkeys in response to  
10 gp100 antigens.

**Materials and Methods**

Plates: MILLIPORE Multiscreen HA plate / MAHA S45.10 (96 wells).

Capture antibodies: MABTECH monoclonal anti-IFN $\gamma$  antibodies/G-Z4 1 mg/mL.

15 Detection antibodies: MABTECH monoclonal anti-IFN $\gamma$  antibodies/7-B6-1-biotin 1 mg/mL.

Enzyme: SIGMA, Extravidin-PA conjugate/E2636

Substrate: BIORAD, NBT/BCIP - Alkaline phosphatase conjugate substrate kit/ref: 170-64 32.

20 **Coating**

Place 100  $\mu$ L per well of capture antibodies at 1  $\mu$ g/mL diluted at 1/1000 in carbonate bicarbonate buffer 0.1M pH 9.6 into the multiwell plate. Incubate overnight at 4°C. Wash 4 times in 1X PBS.

**Saturation**

25 Place 200  $\mu$ L per well of RPMI supplemented with 10% FCS, non essential amino acids, pyruvate, Hepes buffer and Peni-Strepto. Incubate 2 hours at 37°C.

**Test**

Cells from the immunized animals are tested against (a) medium alone; (b) pooled peptides at a concentration of 1 mg/mL; and (c) a non specific stimulus  
30 (PMA-Iono). The pooled peptides used in this Example to stimulate IFN- $\gamma$

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production were derived from gp100 and are illustrated in Tables 5 to 8. The final volume of each sample is 200  $\mu$ L. Incubate 20 hours at 37°C.

Wash 4 times in 1X PBS and 0.05% Tween 20.

#### Detection

- 5 Place 100  $\mu$ L per well of detection antibodies at 1  $\mu$ g/mL diluted in 1/1000 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 2 hours at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

#### Reaction

- Place 100  $\mu$ L per well of Extravidin-PA conjugate diluted 1/6000 in 1X PBS, 1%  
10 BSA and 0.05% Tween 20. Incubate 45 minutes at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

#### Substrate Addition

- Place 100  $\mu$ L per well of substrate previously prepared. For example, for 1 plate, prepare: 9.6 mL of distilled water, 0.4 mL of 25X buffer, 0.1 mL of solution A  
15 (NBT) and 0.1 mL of solution B (BCIP). Incubate 30-45 minutes at room temperature. Wash in distilled water. Dry and transfer to a plastic film. The number of spots are counted using a Zeiss image analyzer. Each spot corresponds to an individual IFN- $\gamma$  secreting T cell.

#### Results

- 20 The results of the ELISPOT analysis are shown in Figures 8-11. The results demonstrate that of the animals tested, 2 out of 2 (i.e. 100%) of the animals that received the intranodal administration of the gp100 antigen, and 2 out of 4 (i.e. 50%) of the animals that received the subcutaneous administration of the gp100 antigen had a positive cell mediated immune response.

#### 25 ELISA Analysis

- The ELISA was performed utilizing standard methodology known in the art. Briefly, the human gp100 ("hgp100"; produced in Baculovirus) was diluted in coating buffer (carbonate-bicarbonate, pH9.6) and added to 96 wells at 0.5 $\mu$ g/well. Plates were placed at 4°C overnight. Plates were then washed and  
30 blocking buffer (phosphate buffered saline/0.5% Tween 20/1.0% BSA, pH7.2)



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was added for 2 hours at 37°C. The plates were then washed and the sera was diluted in dilution buffer (phosphate buffered saline/0.5 % Tween 20/ 0.1 BSA, pH7.2). For this study, monkey sera was diluted to 1:800 and "7" serial 3 fold dilutions were done for each sample tested. The human sera controls were  
5 diluted to 1:50 in dilution buffer and "7" serial 2 fold dilutions were performed. Each dilution was done in duplicate. The plates were incubated a further 2 hours at 37°C. The plates were washed and the horse radish peroxidase (HRP)-conjugated anti-human secondary antibody (anti-human Ig whole antibody from sheep (Amersham Life Science, NA933)) diluted 1:100 in dilution buffer was  
10 added to the wells and incubated for 1 hour at 37°C. The plates were washed and OPD (o-phenylenediamine dihydrochloride) substrate with H<sub>2</sub>O<sub>2</sub> in substrate buffer (50mM phosphate/25mM citrate, pH 7.2) was added to the wells. For a kinetics ELISA, the plate was read repeatedly (2 minute intervals for 15 minutes) unstopped (without "stop" buffer). Plates were read at 450nm.

## 15 Results

The results of the above experiment are presented in Table 9 and in Figure 12. The animals of group 2 received intranodal injections of ALVAC(2)-gp100(mod) followed by boosts with the modified gp100 peptides 209(2M) and 290(9V); the animals in group 3 received a subcutaneous injection of the  
20 ALVAC(2) construct followed by peptide boosts; the animals in group 1 received intranodal injections of saline as a control.

As can be seen from Figure 12, both types of injection of the antigens induced a significant humoral response to the antigen.

In summary, the results of this Example demonstrate that injection of a  
25 tumor antigen according to the invention induces both a significant humoral and cell mediated response.

## EXAMPLE 5

This example presents data obtained from human melanoma patients primed with ALVAC(2)-gp100M and boosted with modified gp100 peptides  
30 (g209-2M and g280-9V).

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## Immunization Protocol

5 Patients were immunized subcutaneously in a prime-boost schedule with ALVAC(2)-gp100M ("prime"; lyophilized ALVAC(2)-gp100M resuspended in 1 ml of 0.4% NaCl; 0.5 ml injections (approximately  $0.5 \times 10^{7.09}$  CCID<sub>50</sub> per injection)) and peptides g209-2M and g280-9V ("boost"; 1000µg/peptide in 1 ml total volume per week (0.2 ml/injection per day x 5 days)). All patients: 1) were HLA-A0201 positive; 2) were between 18 and 70 years of age; 3) exhibited pathologically confirmed malignant melanoma; 4) demonstrated immunocompetence by reactivity to at least 2 or more out of 7 Cell Mediated Immunity (CMI) skin tests; 5) had blood hematology and chemistry values within the following ranges:

## I) Hematology:

Hemoglobin	> 100g/L
Granulocytes	> $2.0 \times 10^9$ /L
Lymphocytes	> $1.5 \times 10^9$ /L
Platelets	> $100 \times 10^9$ /L

## II) Chemistry:

Serum creatinine	< 150 µmol/L
Serum total bilirubin	< 30 µmol/L
AST, ALT, and ALP	Must be < 2x the normal upper limit or < 5x the normal upper limit if due to liver metastases.

15

Patients "primed" with ALVAC(2)-gp100M on weeks 1, 4 and 7; "boosted" with peptides on weeks 10 and 13.

**ELISPOT Analysis:** These results are present in Tables 10 and 11. Peripheral Blood Mononuclear Cells ("PBMNC") were isolated by density centrifugation

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over Ficoll gradients. Cells were bulk-cultured at  $3 \times 10^6$ /ml in AIM-V media along with a mixture of g209-2M and g280-9V or the HLA-A\*0201 binding Flu peptide (all at 50  $\mu$ g/ml) for 8 days. IL-2 was added on days 3 and 5 of culture. On day 9, cells were harvested, counted and  $2 \times 10^5$  cells/well plus 50 U/ml IL-2, with and without the respective peptides, were plated in nitrocellulose membrane containing ELISPOT plates that had been precoated with anti-INF- $\gamma$  antibodies. The plates were developed after 48 hours of culture. The numbers reported are the differences between the average of two wells restimulated with peptide and IL-2 and two wells treated only with IL-2.

Responses are the number of spots (counted by the electronic ELISPOT reader but confirmed in most cases by manual counting) per  $2 \times 10^5$  PBMNC. The number of CD8+ T cells was not routinely determined but is typically 2-5-fold less than this number.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein, are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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**TABLE 1**

Analysis of expression of gp100 antigen by ALVAC-gp100M

	Human gp100M			
	Positive Plaques	negative plaques	total # of plaques	% positive
ALVAC std.	0	571	571	0
vCP1584	387	0	387	100
ALVAC gp100mod L	875	11	886	98.7

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**TABLE 2**

Group Number	Route of administration	Treatment days and compound administered	Number of Animals
1	Intranodal	Saline (NaCl 0.9%): days 28, 42, 56 Then 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
2	Intranodal	ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
3	Subcutaneous	Saline (NaCl 0.9%): day 1 ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70 and 84	4

\*209(2M)-IMDQVPFSY (SEQ.ID.NO.124); 290(9V) YLEPGPVTV (SEQ.ID.NO.125)

- 5
- Group 1 animals (control) received the control article (saline for injection (NaCl 0.9%)).
  - Group 3 animals received the control article (saline for injection (NaCl 0.9%)) on day 1 only.

**TABLE 3**

Group Number	Dose level	Dose volume (ml/administration)
1	Saline (NaCl 0.9%): 0	0.250
2	Dose: $0.25 \times 10^{7.4}$ CCID 50 ALVAC (2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID50	0.250
	Dose: 200 $\mu$ g (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100 $\mu$ g each)	0.2
3	Saline (NaCl 0.9%)	0.250
	ALVAC(2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID 50	0.250
	Dose: 200 $\mu$ g (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100 $\mu$ g each)	0.2

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**TABLE 4**

<b>Days</b>	<b>Sites used</b>
1 and 28	lower left
42	upper left
56	upper right
70	lower left
84	lower right

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**TABLE 5**

## Peptide Pool #1

Peptide	Sequence	SEQ.ID.NO.
1329	HLAVIGALLAVGATK	SEQ.ID.NO.3
1330	GALLAVGATKVPRNQ	SEQ.ID.NO.4
1331	VGATKVPRNQDWLGV	SEQ.ID.NO.5
1332	VPRNQDWLGVSRLR	SEQ.ID.NO.6
1333	DWLGVSRLRRTKAWN	SEQ.ID.NO.7
1334	SRLRRTKAWNRLYP	SEQ.ID.NO.8
1335	TKAWNRLYPEWTEA	SEQ.ID.NO.9
1336	RQLYPEWTEAQRDC	SEQ.ID.NO.10
1337	EWTEAQRDCWRGGQ	SEQ.ID.NO.11
1338	QRDCWRGGQVSLKV	SEQ.ID.NO.12
1339	WRGGQVSLKVSNDGP	SEQ.ID.NO.13
1340	VSLKVSNDGPTLGA	SEQ.ID.NO.14
1344	IALNFPQSQKVLDPG	SEQ.ID.NO.15
1345	PGSQKVLDPGQVIWV	SEQ.ID.NO.16
1346	VLPDGQVIWVNNTII	SEQ.ID.NO.17
1347	QVIWVNNTIINGSQV	SEQ.ID.NO.18
1348	NNTIINGSQVWGGQP	SEQ.ID.NO.19
1349	NGSQVWGGQPVYPQE	SEQ.ID.NO.20
1350	WGGQPVYPQETDDAC	SEQ.ID.NO.21
1351	VYPQETDDACIFPDG	SEQ.ID.NO.22
1352	TDDACIFPDGGPCPS	SEQ.ID.NO.23
1353	IFPDGGPCPSGWSQ	SEQ.ID.NO.24
1355	GSWSQKRSFVYVWKT	SEQ.ID.NO.25
1356	KRSFVYVWKTWGQYW	SEQ.ID.NO.26
1357	YVWKTWGQYWQVLGG	SEQ.ID.NO.27
1358	WGQYWQVLGGPVSL	SEQ.ID.NO.28
1359	QVLGGPVSLSIGTG	SEQ.ID.NO.29



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**TABLE 6**

## Peptide Pool #2

Peptide	Sequence	SEQ.ID.NO.
1360	PVSGLSIGTGRAMLG	SEQ.ID.NO.30
1361	SIGTGRAMLGTHTME	SEQ.ID.NO.31
1362	RAMLGTHTMEVTVYH	SEQ.ID.NO.32
1363	THTMEVTVYHRRGSR	SEQ.ID.NO.33
1364	VTVYHRRGSRSYVPL	SEQ.ID.NO.34
1365	RRGSRSYVPLAHSSS	SEQ.ID.NO.35
1366	SYVPLAHSSSAFTIT	SEQ.ID.NO.36
1368	AFTITDQVPFSVSVS	SEQ.ID.NO.37
1369	DQVPFSVSVSQLRAL	SEQ.ID.NO.38
1370	SVSVSQLRALDGGNK	SEQ.ID.NO.39
1372	DGGNKHFLRNQPLTF	SEQ.ID.NO.40
1373	HFLRNQPLTFALQLH	SEQ.ID.NO.41
1374	QPLTFALQLHDPSCGY	SEQ.ID.NO.42
1375	ALQLHDPSCGYLAEAD	SEQ.ID.NO.43
1379	DFGDSSGTLISRALV	SEQ.ID.NO.44
1380	STGLISRALVVHTY	SEQ.ID.NO.45
1381	SRALVVHTYLEPGP	SEQ.ID.NO.46
1382	VHTYLEPGPVTAQV	SEQ.ID.NO.47
1383	LEPGPVTAQVVLQAA	SEQ.ID.NO.48
1384	VTAQVVLQAAIPLTS	SEQ.ID.NO.49
1385	VLQAAIPLTSCGSSP	SEQ.ID.NO.50
1386	IPLTSCGSSPVP GTT	SEQ.ID.NO.51
1388	VPGTTDGHRPTAEAP	SEQ.ID.NO.52
1389	DGHRPTAEAPNTTAG	SEQ.ID.NO.53
1390	TAEAPNTTAGQVPTT	SEQ.ID.NO.54
1392	QVPTTEVVGTTPGQA	SEQ.ID.NO.55
1393	EVVGTTPGQAPTAEP	SEQ.ID.NO.56

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**TABLE 7**

## Peptide Pool #3

Peptide	Sequence	SEQ.ID.NO.
1394	TPGQAPTAEPSTGTS	SEQ.ID.NO.57
1395	PTAEPSTGTSVQVPT	SEQ.ID.NO.58
1396	SGTTSVQVPTTEVIS	SEQ.ID.NO.59
1397	VQVPTTEVISTAPVQ	SEQ.ID.NO.60
1398	TEVISTAPVQMPTAE	SEQ.ID.NO.61
1399	TAPVQMPTAESTGMT	SEQ.ID.NO.62
1400	MPTAESTGMTPEKVP	SEQ.ID.NO.63
1401	STGMTPEKVPVSEVM	SEQ.ID.NO.64
1402	PEKVPVSEVMGTTLA	SEQ.ID.NO.65
1403	VSEVMGTTLAEMSTP	SEQ.ID.NO.66
1404	GTTLAEMSTPEATGM	SEQ.ID.NO.67
1405	EMSTPEATGMTPEAEV	SEQ.ID.NO.68
1408	SIVVLSTGTTAAQVTT	SEQ.ID.NO.69
1409	SGTTAAQVTTTEWVE	SEQ.ID.NO.70
1410	AQVTTTEWVETTARE	SEQ.ID.NO.71
1411	TEWVETTARELPIPE	SEQ.ID.NO.72
1412	TTARELPIPEPEGPD	SEQ.ID.NO.73
1413	LPIPEPEGPDASSIM	SEQ.ID.NO.74
1414	PEGPDASSIMSTESI	SEQ.ID.NO.75
1415	ASSIMSTESITGSLG	SEQ.ID.NO.76
1416	STESITGSLGPLLDG	SEQ.ID.NO.77
1417	TGSLGPLLDGTATLR	SEQ.ID.NO.78
1418	PLLDGTATLRRLVKRQ	SEQ.ID.NO.79
1419	TATLRRLVKRQVPLDC	SEQ.ID.NO.80
1420	LVKRQVPLDCVLYRY	SEQ.ID.NO.81
1421	VPLDCVLYRYGSFSV	SEQ.ID.NO.82
1422	VLYRYGSFSVTLDIV	SEQ.ID.NO.83

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**Table 8****Peptide Pool #4**

Peptide	Sequence	SEQ.ID.NO.
1424	TLDIVQGIESAEILQ	SEQ.ID.NO.84
1425	QGIESAEILQAVPSG	SEQ.ID.NO.85
1426	AEILQAVPSGEGDAF	SEQ.ID.NO.86
1427	AVPSGEGDAFELTVS	SEQ.ID.NO.87
1428	EGDAFELTVSCQGGL	SEQ.ID.NO.88
1429	ELTVSCQGGLPKEAC	SEQ.ID.NO.89
1430	CQGGLPKEACMEISS	SEQ.ID.NO.90
1431	PKEACMEISSPGCQP	SEQ.ID.NO.91
1432	MEISSPGCQPPAQRL	SEQ.ID.NO.92
1434	PAQRLCQPVLPSPAC	SEQ.ID.NO.93
1435	CQPVLPSPACQLVLH	SEQ.ID.NO.94
1436	PSPACQLVLHQILKG	SEQ.ID.NO.95
1437	QLVLHQILKGGS GTY	SEQ.ID.NO.96
1441	LADTNSLAVVSTQLI	SEQ.ID.NO.97
1442	SLAVVSTQLIMPGQE	SEQ.ID.NO.98
1443	STQLIMPGQEAGLGQ	SEQ.ID.NO.99
1444	MPGQEAGLGQVPLIV	SEQ.ID.NO.100
1445	AGLGQVPLIVGILLV	SEQ.ID.NO.101
1448	LMAVVLASLIYRRRL	SEQ.ID.NO.102
1450	YRRRLMKQDFSVPQL	SEQ.ID.NO.103
1451	MKQDFSVPQLPHSSS	SEQ.ID.NO.104
1452	SVPQLPHSSSHWLRL	SEQ.ID.NO.105
1453	PHSSSHWLRLPRIFC	SEQ.ID.NO.106
1454	HWLRLPRIFCSCPIG	SEQ.ID.NO.107
1455	PRIFCSCPIGENSPL	SEQ.ID.NO.108

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**TABLE 9**

Monkey #	DAY (mOD/min)			
	0	57	68	96
1	3	5	2	2
2	4	6	12	10
3	7	6	10	8
4	7	6	8	8
5	5	9	20	15
6	11	8	10	12
7	11	23	51	30
8	7	30	70	22
9	1	7	5	3
10	2	6	6	4
11	3	7	14	8
12	6	9	15	6

**TABLE 10****Gp100-specific responses to g209-2M and g280-9V\***

Patient	Pre 1 <sup>st</sup> Injection	Pre 2 <sup>nd</sup> Injection	Pre 3 <sup>rd</sup> Injection	Pre 4 <sup>th</sup> Injection	Pre 5 <sup>th</sup> Injection	4 wks post vaccination
#1	0	0	0	ND	ND	2±1.4
#2	0	14±2.8	54±6.4	16±7.8	ND	ND
#3	0	0	ND	ND	ND	ND
#4	0	0	24±13.4	1±2.1	ND	ND
#5	ND	6±6.4	ND	ND	ND	ND

5

**TABLE 11**10 **Flu-peptide specific responses\***

Patient	Pre 1 <sup>st</sup> Injection	Pre 2 <sup>nd</sup> Injection	Pre 3 <sup>rd</sup> Injection	Pre 4 <sup>th</sup> Injection	Pre 5 <sup>th</sup> Injection	4 wks post vaccination
#1	>150	ND	>70	ND	ND	12.5
#2	ND	0	24	0	ND	ND
#3	23.5	7	ND	ND	ND	ND
#4	0	29	13.5	11.5	ND	ND
#5	ND	>200	ND	ND	ND	ND

\* ND signifies that the values were not determined for the sample.

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## WE CLAIM:

1. An isolated and purified modified gp100 molecule capable of modulating an immune response in an animal.
- 5 2. A molecule according to claim 1 having a nucleic acid sequence shown in Figure 1 (SEQ.ID.NO.1).
3. A molecule according to claim 1 or 2 which comprises:
  - 10 (a) a nucleic acid sequence as shown in Figure 1 (SEQ.ID.NO.1) wherein T can also be U;
  - (b) nucleic acid sequences complementary to (a);
  - (c) nucleic acid sequences which are homologous to (a) or (b);
  - (d) a fragment of (a) to (c);
  - 15 (e) a nucleic acid which will hybridize to (a) to (d) under stringent hybridization conditions; and
  - (f) a nucleic acid molecule differing from any of the nucleic acids of (a) to (d) in codon sequences due to the degeneracy of the genetic code.
- 20 4. The nucleic acid of any one of claims 1-3 wherein the nucleic acid is selected from the group consisting of viral nucleic acid, plasmid, bacterial DNA, naked/free DNA, and RNA.
5. A viral nucleic acid of claim 4 wherein the virus is selected from  
25 adenovirus, alphavirus or poxvirus.
6. A poxvirus of claim 5 which is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
- 30 7. The poxvirus of claim 6 which is ALVAC.

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8. A composition comprising the nucleic acid of any one of claims 1-7 and a pharmaceutically acceptable diluent or carrier.
- 5 9. A composition according to claim 8 further comprising an adjuvant.
10. A cell comprising a nucleic acid according to any one of claims 1-7 wherein the cell expresses a polypeptide encoded by the nucleic acid.
- 10 11. A cell according to claim 10 wherein the cell is an antigen-presenting cell.
12. A cell according to claim 10 wherein the cell is a dendritic cell.
13. A recombinant virus comprising a virus into which is inserted a nucleic  
15 acid according to any one of claims 1-7 wherein the nucleic acid encodes for a polypeptide, the recombinant virus causing the expression of the polypeptide in an infected cell.
14. A recombinant virus into which is inserted a nucleic acid according to  
20 any one of claims 1-7 wherein the nucleic acid encodes for a polypeptide, wherein cells infected with the said recombinant virus are capable of eliciting an immune response directly against a member selected from the group consisting of:
- 25 (1) the polypeptide;  
(2) a fragment of the polypeptide;  
(3) a cell expressing the polypeptide or a fragment thereof; or  
(4) cells binding the protein or fragment thereof.
15. A recombinant virus according to claim 13 or 14 selected from  
30 adenovirus, alphavirus, or poxvirus.

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16. A recombinant virus according to claim 15 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
- 5 17. A recombinant virus according to claim 16 wherein the virus is ALVAC.
18. A composition comprising a recombinant virus of any one of claims 13 to 17 and a pharmaceutically acceptable diluent or carrier.
- 10 19. An isolated protein encoded by a nucleic acid molecule according to any one of claims 1-7.
20. An isolated protein having the activity of a modified gp100 protein.
- 15 21. A protein having the amino acid sequence shown in Figure 2 (SEQ.ID.NO.2).
22. A method of modulating an animal's immune system comprising administering an effective amount of a gp100 or gp100 which has been modified.
- 20 23. A method according to claim 22 where the gp100 is gp100M.
24. A method according to claim 22 wherein the gp100 is gp100M.
- 25 25. A method according to claim 24 wherein the gp100M has a nucleic acid sequence as shown in Figure 1 (SEQ.ID.NO.1).
26. A method according to claim 23 wherein the gp100M has an amino acid shown in Figure 2 (SEQ.ID.NO.2).

30



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27. A method of modulating an animal's immune system comprising administering to an animal in need thereof, an effective amount of a vector, into which has been inserted a *gp100* which has been modified, thereby modulating the animal's immune system.
- 5
28. A method according to claim 27 wherein the vector is administered with a lymphokine, cytokine, or a co-stimulatory molecule.
29. A method according to claim 28 wherein the cytokine is GM-CSF, IL-2,  
10 IL-12, TNF, or IFN $\gamma$ 1.
30. A method according to claim 28 wherein the molecule is a lymphokine.
31. A method according to claim 28 wherein the molecule is co-stimulatory  
15 molecule.
32. A method according to claim 31 wherein the co-stimulatory molecule is a molecule of the B7 family.
- 20 33. A method according to any one of claims 27-32 wherein the vector is an adenovirus, alphavirus or poxvirus.
34. A method according to claim 33 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.  
25
35. A method according to claim 34 wherein the poxvirus is ALVAC
36. A method for prophylactic treatment of cancer comprising administering to an animal an effective amount of a modified *gp100* or

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immunogenic fragment thereof, or a nucleic acid sequence encoding a modified gp100 or immunogenic fragment thereof.

37. A method according to claim 36 wherein the modified gp100 has an amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

38. A method according to claim 36 wherein the nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

39. A method according to any one of claims 36, 37 or 38 wherein the cancer is a melanoma.

40. A melanoma vaccine comprising a nucleic acid sequence encoding a modified gp100.

41. A vaccine according to claim 40 wherein the modified gp100 is gp100M.

42. A vaccine according to claim 41 wherein the gp100M has the amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

43. A modified gp100 protein sequence which is modified to enhance its binding to MHC molecules.

44. A modified protein sequence according to claim 43 wherein the protein is gp100M.

45. The protein of claim 44 wherein the amino acid sequence is as shown in Figure 2 (SEQ.ID.NO.2).

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46. A vaccine comprising a modified *gp100* nucleic acid sequence or its corresponding protein or protein fragment capable of eliciting the production of antibodies in a animal to corresponding antigens.
- 5 47. A vaccine according to claim 46 wherein the protein corresponding to the nucleic acid sequence is gp100M.
48. A vaccine according to claim 46 wherein the modified *gp100* nucleic acid sequence is *gp100M*.
- 10 49. A vaccine according to claim 48 wherein the *gp100M* nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).
50. A vaccine according to claim 47 wherein the gp100M has an amino acid  
15 sequence as shown in Figure 2 (SEQ.ID.NO.2).
51. A vaccine comprising a modified *gp100* nucleic acid sequence or its corresponding protein or protein fragment capable of eliciting a cellular immune response.
- 20 52. A vaccine according to claim 51 wherein the protein corresponding to the nucleic acid sequence is gp100M.
53. A vaccine according to claim 51 wherein the modified *gp100* nucleic acid  
25 sequence is *gp100M*.
54. A vaccine according to claim 53 wherein the *gp100M* nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

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55. A vaccine according to claim 52 wherein the gp100M has an amino acid sequence is as shown in Figure 2 (SEQ.ID.NO.2).
56. An immunogenic composition containing a vaccine vector encoding for  
5 a modified gp100 molecule.
57. A composition according to claim 56 wherein the modified gp100 molecule is gp100M.
- 10 58. A composition according to claim 57 wherein the modified gp100M has an amino acid sequence according to Figure 2 (SEQ.ID.NO.2).
59. A composition according to any one of claims 56, 57 or 58 wherein the vector is an adenovirus, alphavirus or poxvirus.  
15
60. A composition according to claim 59 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
61. A composition according to claim 60 wherein the poxvirus is ALVAC.  
20
62. Immunogenic fragments of an isolated gp100M protein encoded by a nucleic acid molecule having a sequence according to SED ID NO. 1.

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## FIGURE 1

	ATGG	ATCTGGTGCT	AAAAAGATGC	CTTCTTCATT	TGGCTGTGAT
AGGTGCTTTE	CTGGCTGTGG	GGGCTACAAA	AGTACCCAGA	AACCAGGACT	GGCTTGGTGT
CTCAAGGCAA	CTCAGAACCA	AAGCCTGGAA	CAGGCAGCTG	TATCCAGAGT	GGACAGAAGC
CCAGAGACTT	GACTGCTGGA	GAGGTGGTCA	AGTGTCCCTC	AAGGTCAGTA	ATGATGGGCC
TACACTGATT	GGTGCAAATG	CCTCCTTCTC	TATTGCCTTG	AACTTCCCTG	GAAGCCAAAA
GGTATTGCCA	GATGGGCAGG	TTATCTGGGT	CAACAATACC	ATCATCAATG	GGAGCCAGGT
GTGGGGAGGA	CAGCCAGTGT	ATCCCCAGGA	AACTGACGAT	GCCTGCATCT	TCCTGATGG
TGGACCTTGC	CCATCTGGCT	CTTGGTCTCA	GAAGAGAAGC	TTTGTTTATG	TCTGGAAGAC
CTGGGGCCAA	TACTGGCAAG	TTCTAGGGGG	CCCAGTGTCT	GGGCTGAGCA	TTGGGACAGG
CAGGCCAATG	CTGGGCACAC	ACACGATGGA	AGTCACTGTC	TACCATCGCC	GGGGATCCCG
GAGCTATGTG	CCTCTTGCTC	ATTCCAGCTC	AGCCTTCACC	ATTATGGACC	AGGTGCCTTT
CTCCGTGAGC	GTGTCCCAGT	TGCGGGCCTT	GGATGGAGGG	AACAGCACT	TCCTGAGAAA
TCAGCCTCTG	ACCTTTGCCC	TCCAGCTCCA	TGACCCAGT	GGCTATCTGG	CTGAAGCTGA
CCTCTCCTAC	ACCTGGGACT	TTGGAGACAG	TAGTGGAACC	CTGATCTCTC	GGGCACTTGT
GGTCACTCAT	ACTTACCTGG	AGCCTGGCCC	AGTCACTGTT	CAGGTGGTCC	TGCAGGCTGC
CATTCTCTC	ACCTCCTGTG	GCTCCTCCCC	AGTTCCAGGC	ACCACAGATG	GGCACAGGCC
AACTGCAGAG	GCCCCAACA	CCACAGCTGG	CCAAGTGCCT	ACTACAGAAG	TTGTGGGTAC
TACACCTGGT	CAGGCGCCAA	CTGCAGAGCC	CTCTGGAACC	ACATCTGTGC	AGGTGCCAAC
CACTGAAGTC	ATAAGCACTG	CACCTGTGCA	GATGCCAACT	GCAGAGAGCA	CAGGTATGAC
ACCTGAGAAG	GTGCCAGTTT	CAGAGGTCAT	GGGTACCACA	CTGGCAGAGA	TGTCAACTCC
AGAGGCTACA	GGTATGACAC	CTGCAGAGGT	ATCAATTGTG	GTGCTTTCTG	GAACCACAGC
TGCACAGGTA	ACAACTACAG	AGTGGGTGGA	GACCACAGCT	AGAGAGCTAC	CTATCCCTGA
GCCTGAAGST	CCAGATGCCA	GCTCAATCAT	GTCTACGGAA	AGTATTACAG	GTTCCCTGGG
CCCCCTGCTG	GATGGTACAG	CCACCTTAAG	GCTGGTGAAG	AGACAAGTCC	CCCTGGATTG
TGTTCTGTAT	CGATATGGTT	CCTTTTCCGT	CACCCTGGAC	ATTGTCCAGG	GTATTGAAAG
TGCCGAGATC	CTGCAGGCTG	TGCCGTCCGG	TGAGGGGGAT	GCATTTGAGC	TGACTGTGTC
CTGCCAAGGC	GGGCTGCCCA	AGGAAGCCTG	CATGGAGATC	TCATCGCCAG	GGTGCCAGCC
CCCTGCCCAG	CGGCTGTGCC	AGCCTGTGCT	ACCCAGCCCA	GCCTGCCAGC	TGGTTCTGCA
CCAGATACTG	AAGGGTGGCT	CGGGGACATA	CTGCCTCAAT	GTGTCTCTGG	CTGATACCAA
CAGCCTGGCA	GTGGTCAGCA	CCCAGCTTAT	CATGCCTGGT	CAAGAAGCAG	GCCTTGGGCA
GGTTCCGCTG	ATCGTGGGCA	TCTTGCTGGT	GTTGATGGCT	GTGGTCCTTG	CATCTCTGAT
ATATAGGCGC	AGACTTATGA	AGCAAGACTT	CTCCGTACCC	CAGTTGCCAC	ATAGCAGCAG
TCACTGGCTG	CGTCTACCCC	GCATCTTCTG	CTCTTGTCCT	ATTGGTGAGA	ACAGCCCCCT
CCTCAGTGGG	CAGCAGGTCT	GA			

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## FIGURE 2

Met	Asp	Leu	Val	Leu	Lys	Arg	Cys	Leu	Leu	His	Leu	Ala	Val	Ile	Gly	1	5	10	15
Ala	Leu	Leu	Ala	Val	Gly	Ala	Thr	Lys	Val	Pro	Arg	Asn	Gln	Asp	Trp	20	25	30	
Leu	Gly	Val	Ser	Arg	Gln	Leu	Arg	Thr	Lys	Ala	Trp	Asn	Arg	Gln	Leu	35	40	45	
Tyr	Pro	Glu	Trp	Thr	Glu	Ala	Gln	Arg	Leu	Asp	Cys	Trp	Arg	Gly	Gly	50	55	60	
Gln	Val	Ser	Leu	Lys	Val	Ser	Asn	Asp	Gly	Pro	Thr	Leu	Ile	Gly	Ala	65	70	75	80
Asn	Ala	Ser	Phe	Ser	Ile	Ala	Leu	Asn	Phe	Pro	Gly	Ser	Gln	Lys	Val	85	90	95	
Leu	Pro	Asp	Gly	Gln	Val	Ile	Trp	Val	Asn	Asn	Thr	Ile	Ile	Asn	Gly	100	105	110	
Ser	Gln	Val	Trp	Gly	Gly	Gln	Pro	Val	Tyr	Pro	Gln	Glu	Thr	Asp	Asp	115	120	125	
Ala	Cys	Ile	Phe	Pro	Asp	Gly	Gly	Pro	Cys	Pro	Ser	Gly	Ser	Trp	Ser	130	135	140	
Gln	Lys	Arg	Ser	Phe	Val	Tyr	Val	Trp	Lys	Thr	Trp	Gly	Gln	Tyr	Trp	145	150	155	160
Gln	Val	Leu	Gly	Gly	Pro	Val	Ser	Gly	Leu	Ser	Ile	Gly	Thr	Gly	Arg	165	170	175	
Ala	Met	Leu	Gly	Thr	His	Thr	Met	Glu	Val	Thr	Val	Tyr	His	Arg	Arg	180	185	190	
Gly	Ser	Arg	Ser	Tyr	Val	Pro	Leu	Ala	His	Ser	Ser	Ser	Ala	Phe	Thr	195	200	205	
Ile	Met	Asp	Gln	Val	Pro	Phe	Ser	Val	Ser	Val	Ser	Gln	Leu	Arg	Ala	210	215	220	
Leu	Asp	Gly	Gly	Asn	Lys	Phe	Leu	Arg	Asn	Gln	Pro	Leu	Thr	Phe		225	230	235	240
Ala	Leu	Gln	Leu	His	Asp	Pro	Ser	Gly	Tyr	Leu	Ala	Glu	Ala	Asp	Leu	245	250	255	
Ser	Tyr	Thr	Trp	Asp	Phe	Gly	Asp	Ser	Ser	Gly	Thr	Leu	Ile	Ser	Arg	260	265	270	
Ala	Leu	Val	Val	Thr	His	Thr	Tyr	Leu	Glu	Pro	Gly	Pro	Val	Thr	Val	275	280	285	
Gln	Val	Val	Leu	Gln	Ala	Ala	Ile	Pro	Leu	Thr	Ser	Cys	Gly	Ser	Ser	290	295	300	
Pro	Val	Pro	Gly	Thr	Thr	Asp	Gly	His	Arg	Pro	Thr	Ala	Glu	Ala	Pro	305	310	315	320
Asn	Thr	Thr	Ala	Gly	Gln	Val	Pro	Thr	Thr	Glu	Val	Val	Gly	Thr	Thr	325	330	335	
Pro	Gly	Gln	Ala	Pro	Thr	Ala	Glu	Pro	Ser	Gly	Thr	Thr	Ser	Val	Gln	340	345	350	
Val	Pro	Thr	Thr	Glu	Val	Ile	Ser	Thr	Ala	Pro	Val	Gln	Met	Pro	Thr	355	360	365	

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## FIGURE 2 (CONT'D)

Ala	Glu	Ser	Thr	Gly	Met	Thr	Pro	Glu	Lys	Val	Pro	Val	Ser	Glu	Val
370						375					380				
Met	Gly	Thr	Thr	Leu	Ala	Glu	Met	Ser	Thr	Pro	Glu	Ala	Thr	Gly	Met
385					390					395					400
Thr	Pro	Ala	Glu	Val	Ser	Ile	Val	Val	Leu	Ser	Gly	Thr	Thr	Ala	Ala
				405					410					415	
Gln	Val	Thr	Thr	Thr	Glu	Trp	Val	Glu	Thr	Thr	Ala	Arg	Glu	Leu	Pro
			420					425					430		
Ile	Pro	Glu	Pro	Glu	Gly	Pro	Asp	Ala	Ser	Ser	Ile	Met	Ser	Thr	Glu
		435					440					445			
Ser	Ile	Thr	Gly	Ser	Leu	Gly	Pro	Leu	Leu	Asp	Gly	Thr	Ala	Thr	Leu
	450					455					460				
Arg	Leu	Val	Lys	Arg	Gln	Val	Pro	Leu	Asp	Cys	Val	Leu	Tyr	Arg	Tyr
465					470					475					480
Gly	Ser	Phe	Ser	Val	Thr	Leu	Asp	Ile	Val	Gln	Gly	Ile	Glu	Ser	Ala
				485					490					495	
Glu	Ile	Leu	Gln	Ala	Val	Pro	Ser	Gly	Glu	Gly	Asp	Ala	Phe	Glu	Leu
			500					505					510		
Thr	Val	Ser	Cys	Gln	Gly	Gly	Leu	Pro	Lys	Glu	Ala	Cys	Met	Glu	Ile
		515					520					525			
Ser	Ser	Pro	Gly	Cys	Gln	Pro	Pro	Ala	Gln	Arg	Leu	Cys	Gln	Pro	Val
	530					535					540				
Leu	Pro	Ser	Pro	Ala	Cys	Gln	Leu	Val	Leu	His	Gln	Ile	Leu	Lys	Gly
545					550					555					560
Gly	Ser	Gly	Thr	Tyr	Cys	Leu	Asn	Val	Ser	Leu	Ala	Asp	Thr	Asn	Ser
				565					570					575	
Leu	Ala	Val	Val	Ser	Thr	Gln	Leu	Ile	Met	Pro	Gly	Gln	Glu	Ala	Gly
			580				585						590		
Leu	Gly	Gln	Val	Pro	Leu	Ile	Val	Gly	Ile	Leu	Leu	Val	Leu	Met	Ala
	595					600						605			
Val	Val	Leu	Ala	Ser	Leu	Ile	Tyr	Arg	Arg	Arg	Leu	Met	Lys	Gln	Asp
	610					615					620				
Phe	Ser	Val	Pro	Gln	Leu	Pro	His	Ser	Ser	Ser	His	Trp	Leu	Arg	Leu
625					630					635					640
Pro	Arg	Ile	Phe	Cys	Ser	Cys	Pro	Ile	Gly	Glu	Asn	Ser	Pro	Leu	Leu
				645					650					655	
Ser	Gly	Gln	Gln	Val											
				660											

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## FIGURE 3

## Nucleotide Sequence of C5H6gp100M

1-254 left C5 flanking arm  
 255-376 H6 promoter  
 377-2362 modified gp100 gene  
 2363-2534 right C5 flanking arm

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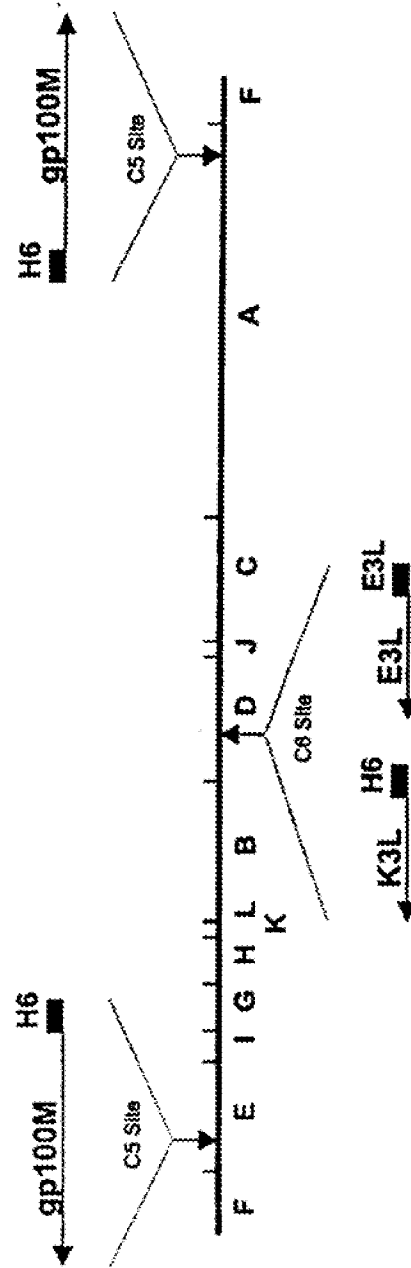
1  GGCTACTTTT CAACAAAGGA GCAGATGTAA ACTACATCTT TGAAAGAAAT GGAAAATCAT
61 ATACTGTTTT GGAATTGATT AAAGAAAGTT ACTCTGAGAC ACAAAGAGG TAGCTGAAGT
121 GGTACTCTCA AAGGTACGTG ACTAATTAGC TATAAAAAGG ATCGTCGACG AGCTCGAATT
181 CGGATCCGGG TTAATTAATT AGTCATCAGG CAGGGCGAGA ACGAGACTAT CTGCTCGTTA
241 ATTAATTAGA GCTTCTTTAT TCTATACTTA AAAAGTGAAA ATAAATACAA AGGTTCTTGA
301 GGGTTGTGTT AATTGAAAG CGAGAAATAA TCATAAATTA TTTTATTATC GCGATATCCG
361 TTAAGTTTGT ATCGTAATGG ATCTGGTGCT AAAAGATGC CTTCTTCATT TGGCTGTGAT
421 AGGTGCTTTG CTGGCTGTGG GGGCTACAAA AGTACCCAGA AACCAGGACT GGCTGGGTGT
481 CTCAGGCAA CTCAGAACCA AAGCCTGGAA CAGGCAGCTG TATCCAGAGT GGACAGAAGC
541 CCAGAGACTT GACTGCTGGA GAGGTGGTCA AGTGTCCCTC AAGGTCAGTA ATGATGGGCC
601 TACACTGATT GGTGCAAATG CCTCCTTCTC TATTGCCITG AACTTCCCTG GAAGCCAAAA
661 GGTATTGCCA GATGGGCAGG TTATCTGGGT CAACAATACC ATCATCAATG GGAGCCAGGT
721 GTGGGGAGGA CAGCCAGTGT ATCCCCAGGA AACTGACGAT GCCTGCATCT TCCCTGATGG
781 TGGACCTTGC CCATCTGGCT CTTGGTCTCA GAAGAGAAGC TTTGTTTATG TCTGGAAGAC
841 CTGGGGCCAA TACTGGCAAG TTCTAGGGGG CCCAGTGTCT GGGCTGAGCA TTGGGACAGG
901 CAGGGCAATG CTGGGCACAC ACACGATGGA AGTGAAGTGC TACCATCGCC GGGGATCCCG
961 GAGCTATGTG CCTCTTGCTC ATTCCAGCTC AGCCTTCACC ATTATGGACC AGGTGCCTTT
1021 CTCCGTGAGC GTGTCCCAGT TGCGGGCCTT GGATGGAGGG AACAAGCACT TCCTGAGAAA
1081 TCAGCCTCTG ACCTTTGCCC TCCAGCTCCA TGACCCAGT GGCTATCTGG CTGAAGCTGA
1141 CCTCTCCTAC ACCTGGGACT TTGGAGACAG TAGTGGAAAC CTGATCTCTC GGGCACTTGT
1201 GGTCACTCAT ACTTACCTGG AGCCTGGCCC AGTCACTGTT CAGGTGGTCC TGCAGGCTGC
1261 CATTCTCTCT ACCTCCTGTG GCTCCTCCCC AGTTCCAGGC ACCACAGATG GGCACAGGCC
1321 AACTGCAGAG GCCCTTAACA CCACAGCTGG CCAAGTGCCT ACTACAGAAG TTGTGGGTAC
1381 TACACTGGGT CAGGCGCCAA CTGCAGAGCC CTCTGGAACC ACATCTGTGC AGGTGCCAAC
1441 CACTGAAGTC ATAAGCACTG CACCTGTGCA GATGCCAACT GCAGAGAGCA CAGGTATGAC
1501 ACCTGAGAAG GTGCCAGTTT CAGAGGTCAT GGGTACCACA CTGGCAGAGA TGTCAACTCC
1561 AGAGGCTACA GGTATGACAC CTGCAGAGGT ATCAATTGTG GTGCTTTCTG GAACCACAGC
1621 TGCACAGGTA ACAACTACAG AGTGGGTGGA GACCACAGCT AGAGAGCTAC CTATCCCTGA
1681 GCCTGAAGGT CCAGATGCCA GCTCAATCAT GTCTACGGAA AGTATTACAG GTTCCCTGGG
1741 CCCCCTGCTG GATGGTACAG CCACCTTAAG GCTGGTGAAG AGACAAGTCC CCCTGGATTG
1801 TGTTCTGTAT CGATATGGTT CCTTTTCCGT CACCCTGGAC ATTGTCCAGG GTATTGAAAG
1861 TGCCGAGATC CTGCAGGCTG TGCCGTCCGG TGAGGGGGAT GCATTTGAGC TGACTGTGTC
1921 CTGCCAAGGC GGGCTGCCCC AGGAAGCCTG CATGCAGATC TCATGCCAGC GGTGCCAGCC
1981 CCCTGCCCAG CGGCTGTGCC AGCCTGTGCT ACCCAGCCCA GCCTCCAGC TGGTTCTGCA
2041 CCAGATACTG AAGGGTGGCT CGGGACATA CTGCCTCAAT GTGTCTCTGG CTGATACCAA
2101 CAGCCTGGCA GTGGTCAGCA CCCAGCTTAT CATGCCCTGT CAAGAAGCAG GCCTTGGGCA
2161 GGTTCGCTG ATCGTGGGCA TCTTGCTGGT GTTGATGGCT GTGGTCCTTG CATCTCTGAT
2221 ATATAGGCGC AGACTTATGA AGCAAGACTT CTCCGTACCC CAGTTGCCAC ATAGCAGCAG
2281 TCACTGGCTG CGTCTACCCC GCATCTTCTG CTCTTGTCCT ATTGGTGAGA ACAGCCCCCT
2341 CCTCAGTGGG CAGCAGGTCT GATTTTATC TCGAGTCTAG AATCGATCCC GGGTTTTTAT
2401 GACTAGTTAA TCACGCCGCG TTATAAAGAT CTAAAATGCA TAATTTCTAA ATAATGAAAA
2461 AAAAGTACAT CATGAGCAAC GCGTTAGTAT ATTTTACAA GGAGATTAA GCTCTATACC
2521 GTTCTATGTT TATT

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## FIGURE 4

**ALVAC(2)-gp100M (vCP1584)**  
(ALVAC XhoI Restriction Map)

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## FIGURE 5

## Oligonucleotide Primers

IDC5-1

CGT GCC ATG GCA CAC AAA AGA GGT AGC TGA A

IDC5-2

CCA GGC GGC CGC ACT AAC GCG TTG CTC ATG ATG

C5L

CAC AAA AGA GGT AGC TGA AGT

MEL 01

ATG GAT CTG GTG CTA AAA AGA

MEL 05

ACC TTG CCC ATC TGG CTC TTG

MEL 09

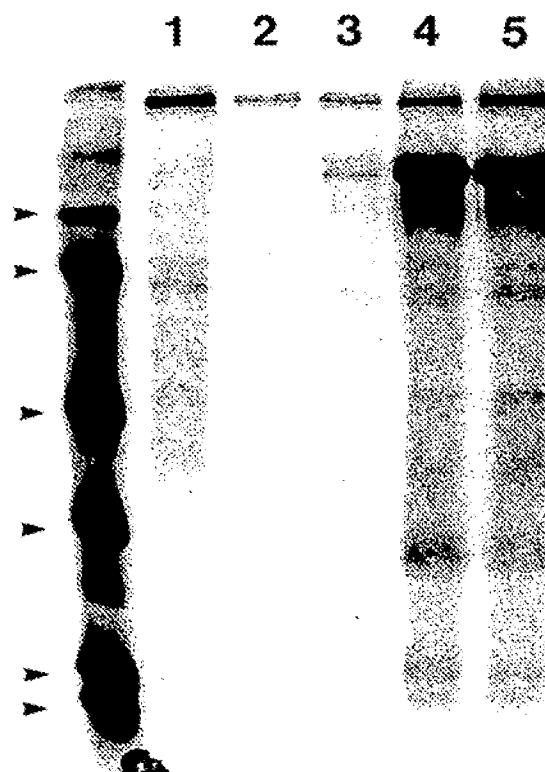
AGA TGC CAG CTC AAT CAT GTG

CSR

ATA GAT CTT TAT AAG CGG CCG

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## FIGURE 6



Molecular Weight Markers: 200, 98.6, 68, 43, 29, 18, 14 kDa

Lane 1: Uninfected HeLa cells

Lane 2: HeLa cells infected with ALVAC

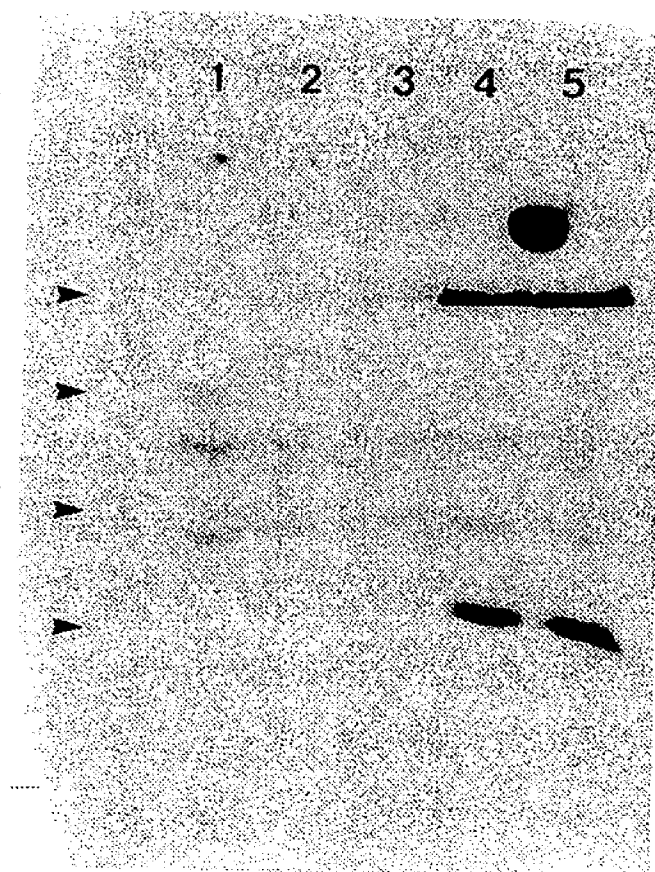
Lane 3: HeLa cells infected with ALVAC-gp100 (vCP1465)

Lane 4: HeLa cells infected with ALVAC(2)-gp100M (vCP1584)

Lane 5: HeLa cells infected with ALVAC(2)-gp100M (sister of vCP1584)

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## FIGURE 7



Molecular Weight Markers: 97, 68, 43, 29 kDa

Lane 1: Uninfected HeLa cells

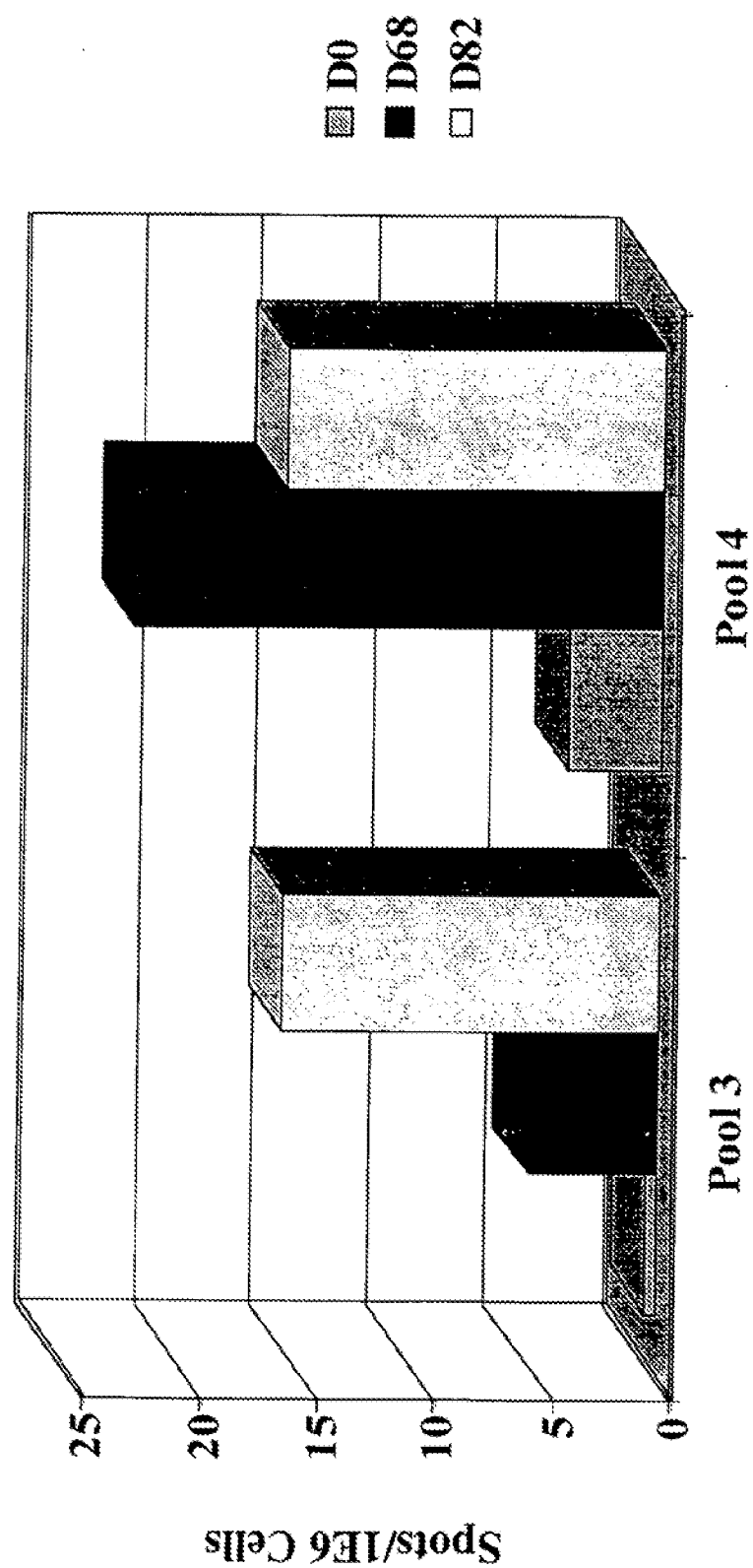
Lane 2: HeLa cells infected with ALVAC

Lane 3: HeLa cells infected with ALVAC-gp100 (vCP1465)

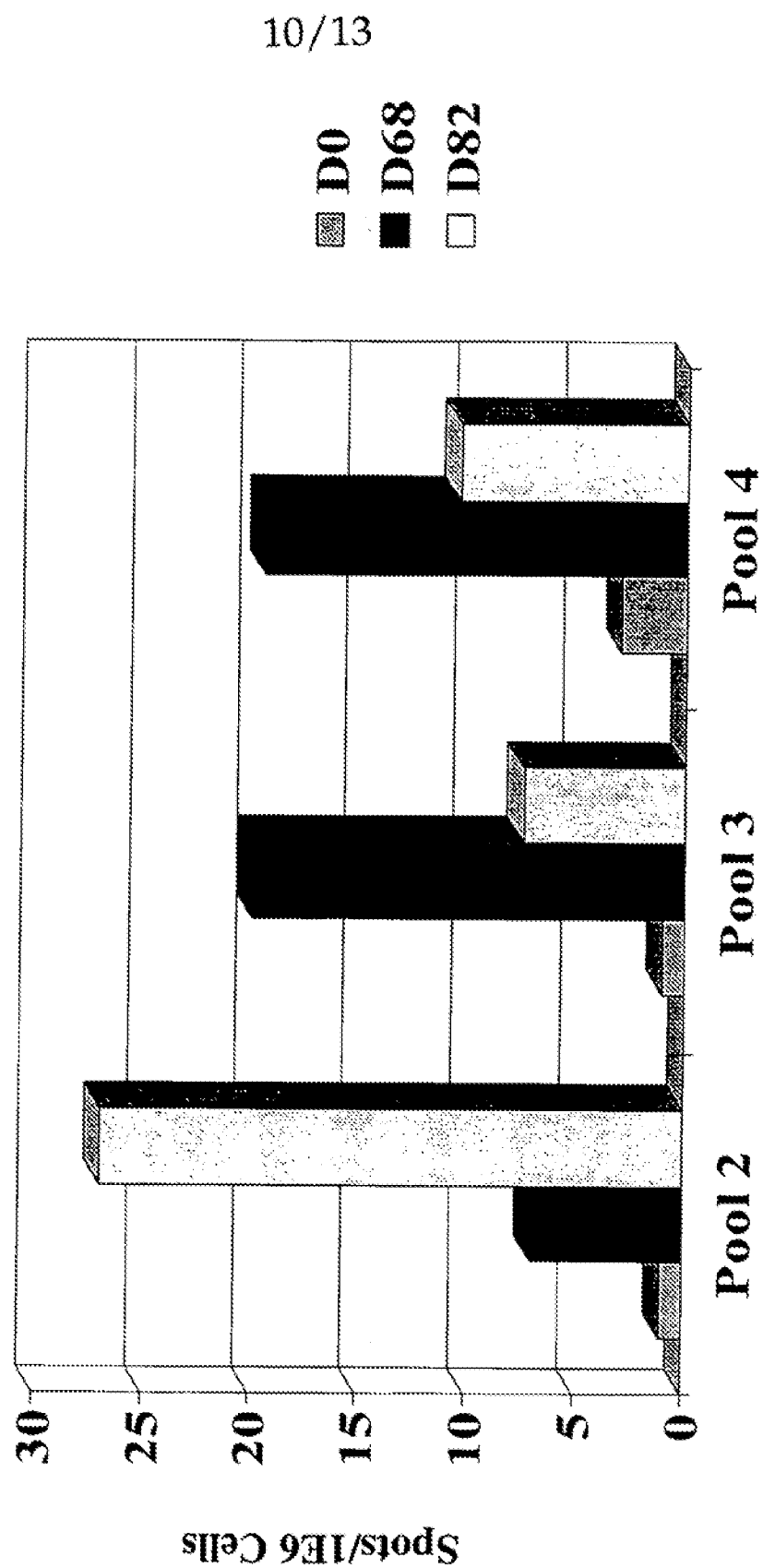
Lane 4: HeLa cells infected with ALVAC(2)-gp100M (vCP1584)

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**FIGURE 8**  
**Monkey #6 (Intranodal Administration)**

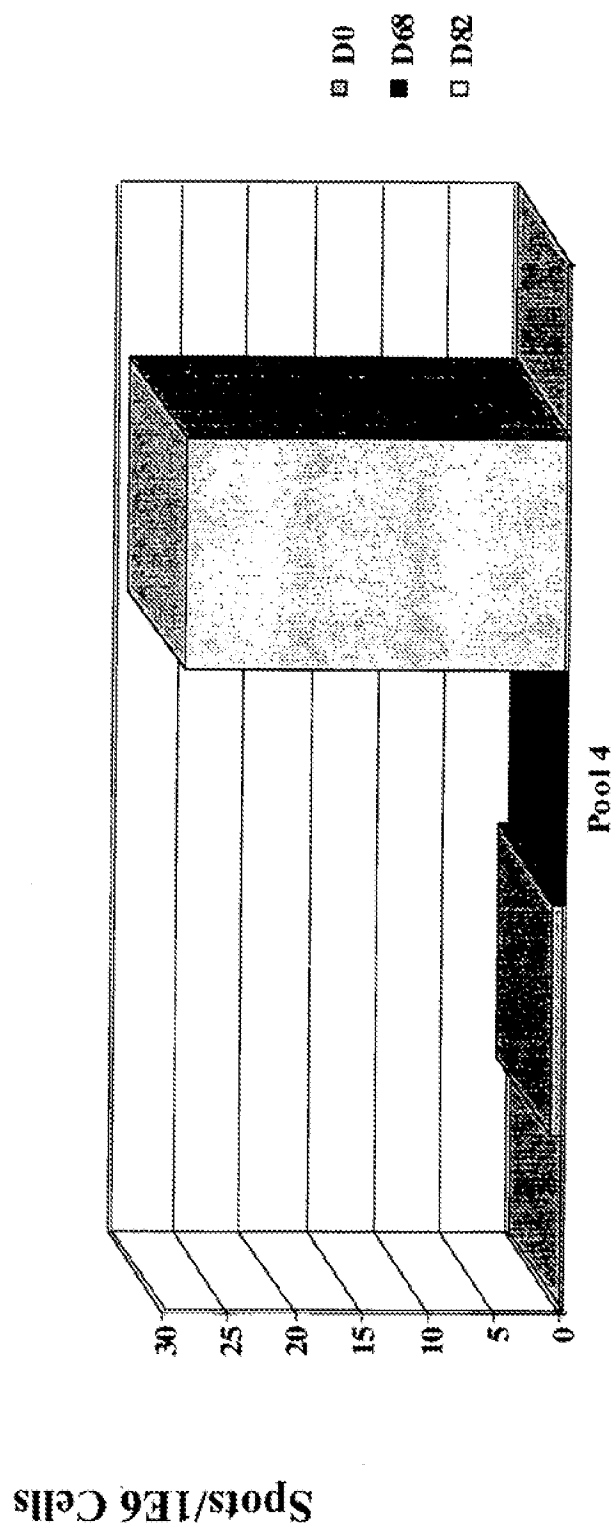


**FIGURE 9**  
**Monkey #7 (Intranodal Administration)**



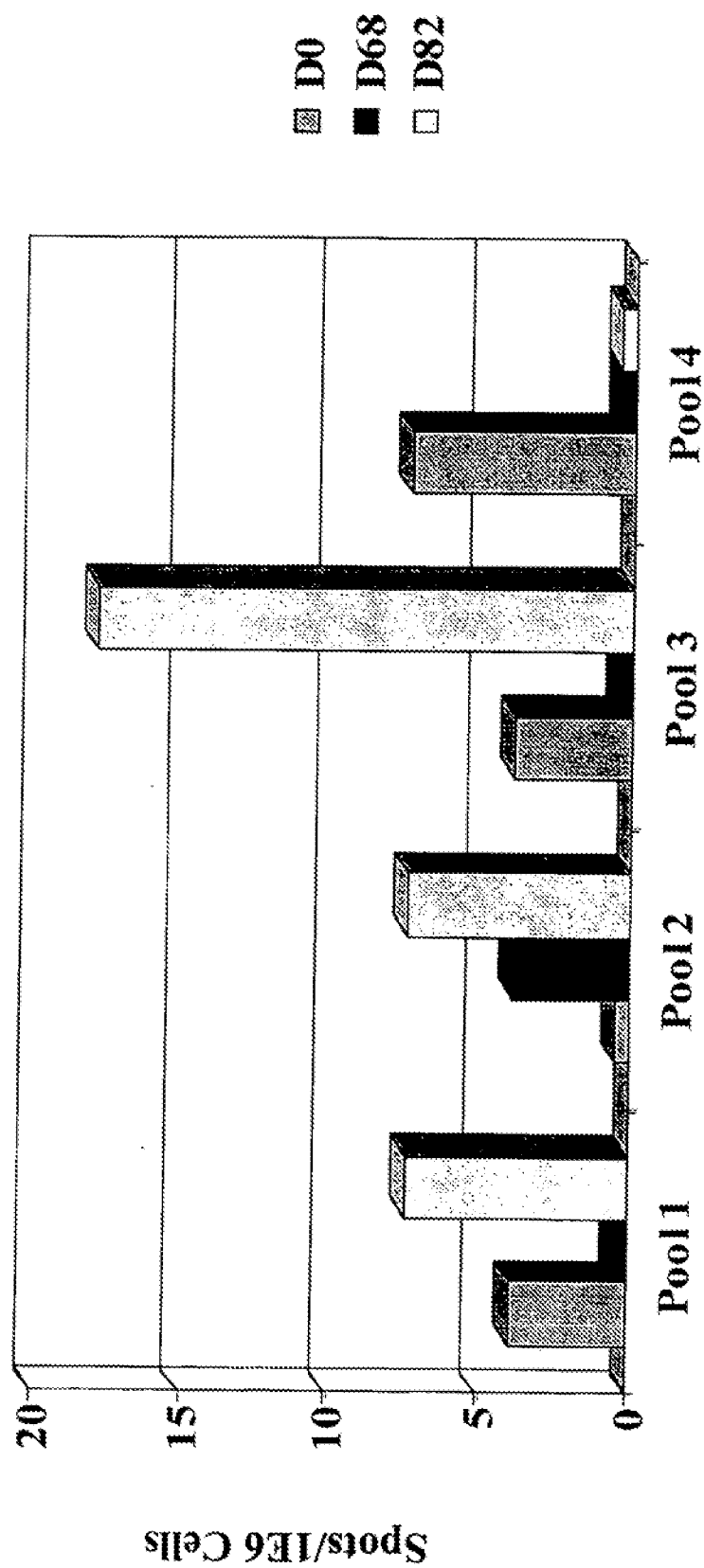
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**FIGURE 10**  
**Monkey # 11 (Subcutaneous Administration)**



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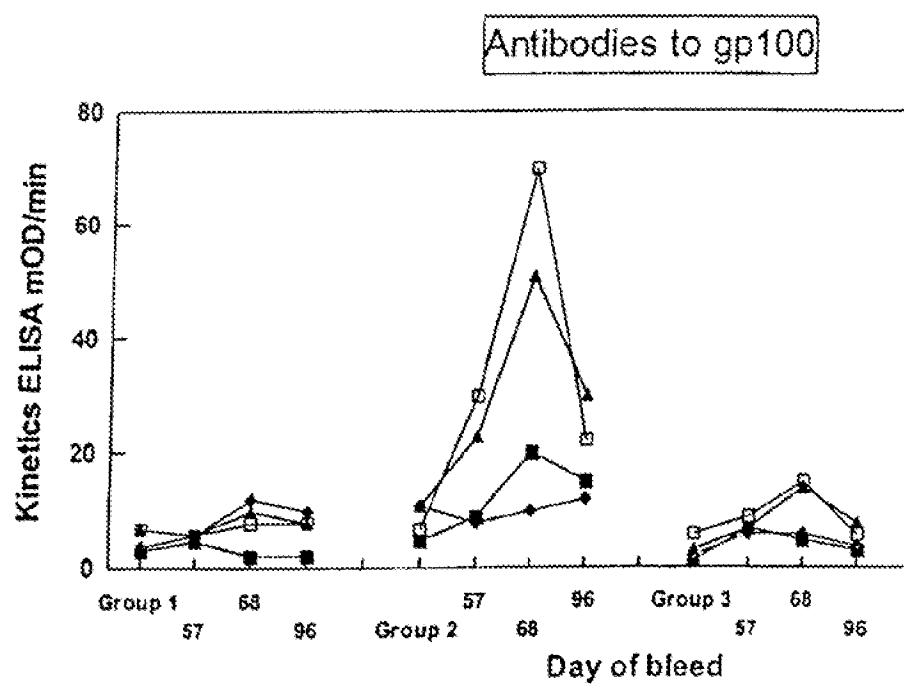
**FIGURE 11**  
**Monkey #10 (Subcutaneous Administration)**





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FIGURE 12



## INTERNATIONAL SEARCH REPORT

National Application No

PCT/CA 00/01254

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/705 C12N9/64 A61K39/00 C12N5/06 C12N7/00  
 A61K35/76 A61P35/00 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, LIFESCIENCES, EMBASE, CHEM ABS Data, SCISEARCH, BIOSIS, WPI  
 Data, EPO-Internal, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>IRVINE KARI R ET AL: "Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens."            CANCER RESEARCH,            vol. 59, no. 11, 1 June 1999 (1999-06-01),            pages 2536-2540, XP002161590            ISSN: 0008-5472</p> <p>page 2536, left-hand column, line 39            -right-hand column, line 14            page 2356, right-hand column, line 47            -page 2357, left-hand column, line 1            table 1</p> <p style="text-align: center;">--- -/--</p>	<p>8,9,            13-16,            18-20,            22-24,            27-34,            36,            39-41,            43,44,            46-48,            51-53,            56,57,            59,60</p>

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

28 February 2001

Date of mailing of the international search report

12/03/2001

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 00/01254

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 02538 A (AKZO NOBEL N.V., NETH.; FIGDOR, CARL GUSTAV; ADEMA, GOSSE JAN) 22 January 1998 (1998-01-22) page 3, line 3-19 page 11, line 15 -page 13, line 13 examples 2-5 -----	1-62
A	WO 98 04728 A (THERION BIOLOG CORP ;US HEALTH (US)) 5 February 1998 (1998-02-05) page 6, line 9 -page 11, line 17 claims -----	1-62
A	PARKHURST M R ET AL: "Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201-binding residues." JOURNAL OF IMMUNOLOGY, (1996 SEP 15) 157 (6) 2539-48. , XP002096010 page 2539, right-hand column, paragraph 2 -page 2340, left-hand column, paragraph 2 -----	1,19-21, 43-45,61
A	WO 99 46992 A (GREGORY RICHARD J ;GENZYME CORP (US); KAPLAN JOHANNE (US)) 23 September 1999 (1999-09-23) claims -----	10-12
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